

RETARDATION OF MUSCLE GROWTH IN CASTRATED MALE MICE: FURTHER EVIDENCE FOR HORMONAL INFLUENCE ON MUSCLE DEVELOPMENT

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SUMMARY

Retardation of muscle growth in castrated male mice was studied as an evidence for the influence of hormones on the development of muscle mass. Male albino mice were castrated at 28 days of age by open castration method. The weights and the muscle mass indices (mg muscle weight per gram body weight) of the muscles of these mice were compared with those of control male and female mice at an adult age of 12 weeks. The results obtained showed that the absolute weights and the muscle mass indices of the muscles of castrated males were significantly smaller ($P < 0.05$) than those of control males. The weights of the muscles of castrated males were significantly greater ($P < 0.01$) than those of control females. However, there were no significant differences ($P > 0.05$) between the muscle mass indices of muscles of control female and castrated male mice. These results indicated that retardation of muscle growth following castration of male mice may be the result of loss of the stimulatory influence of testosterone on muscle development in the castrated male mice.

KEY WORDS: Castration, Testosterone, Muscles, Mice.

INTRODUCTION

Muscles of adult male mice are invariably larger than those of adult female mice (Goldspink and Rowe, 1968). A similar observation was made in rats by Cheek *et al.* (1968). The sex difference was attributed to greater increase in the size of the myofibres in the males, as there was no difference in the number of myofibres in the male and female mice (Rowe and Goldspink, 1968; Hanrahan *et al.*, 1973; Aberle and Doolittle, 1976). Studies by Venable (1966a; 1966b) have shown that replacement therapy using testosterone, following castration of males caused considerable hypertrophy of the fibres in

rat levator ani muscles. It was noted that the hypertrophy of the muscle was a result of increase in myofibrillar material of the fibres. There was no change in the number of fibres either during the replacement therapy or during atrophy of the muscles following castration. At the cellular level, an increased incorporation of labeled amino acids into muscle protein after administration of androgens to both intact and castrated animals have been reported (Buresova *et al.*, 1969). Ribosomes obtained from castrated male animals were less active in synthesizing protein than those obtained from intact animals (Breuer and Florini, 1965). Thus, androgens have been shown to increase

the rate of protein synthesis in most muscles. Goldspink (1972) therefore, suggested that hormones, particularly, androgens might be presumed to have a direct or at least, an indirect stimulatory influence on the development of muscles.

Large doses of oestrogen administered within the first 5 days of post-natal life have been found to produce a depressant effect on the development of various muscles in sexually immature female rabbits (Ihemelandu, 1980), as well as male and female mice (Ihemelandu, 1981; 1984). These observations suggest that the smaller muscle mass observed in mature females when compared to mature male animals may be a result of the inhibitory role of oestrogen on muscle development in the females. Ihemelandu (1988) did not observe any difference between the weights of muscles of prepubertal male and female mice at 4 weeks of age. He, however, reported that the weights of the muscles of the males were greater than those of the females at 5 and 6 weeks of age. According to him, this period coincided with the period of significant increase in testosterone and oestrogen secretion in the males and females respectively, hence, the differences in their muscle weights. It has been demonstrated that oestrogen brought about this inhibition of muscle mass development in the female mice by limiting the sizes of individual muscle fibres, and not by retarding increase in number of muscle fibres (Ihemelandu, 1984). Furthermore, in the presence of oestrogen, there existed no difference between the muscle mass of developing male and female mice (Ihemelandu, 1981).

The present study was designed to further investigate the effect of hormones particularly, testosterone on the development of muscles in mice.

MATERIALS AND METHODS

Experimental animals

Twelve adult female mice and four adult male mice were used as the breeding stock in this study. These were selected from a group of inbred albino mice maintained for research in the Animal house of the Department of Veterinary Anatomy, University of Nigeria, Nsukka. The females in the breeding stock were mated using the males at a ratio of 3 females to 1 male. Following parturition, the litter size for each dam was adjusted to 6 until weaning at 21 days of age. The animals used for this study were randomly selected from the offspring of the breeding stock. 17 males and 17 females were selected to constitute control males and females respectively. 17 other males were randomly selected and castrated at 28 day of age. Castration was by the open castration method of Ihemelandu and Ibebunjo (1992). These experimental animals were housed 3 or 4 per cage according to sex and study group until 12 weeks of age. They were maintained on a commercially prepared diet and drinking water *ad libitum*. At 12 weeks of age, each mouse was sacrificed by severing the spinal cord at the atlanto-occipital joint. This age was chosen because the muscles of male and female mice attain mature weights at 12 to 15 weeks and 9 weeks of age respectively (Rowe and Goldspink, 1969).

Quantitative measurements

Prior to sacrificing each mouse, the live body weight was determined. Following death, two forelimb muscles namely, Biceps brachii and Triceps brachii muscles, as well as two hind limb muscles namely, Soleus and Gastrocnemius muscles were carefully dissected out and weighed. The mean weight of the right and left muscles was determined and used as weight for the particular muscle. The muscle mass index (milligram muscle weight per gram body weight) was also determined for each muscle. This allometric parameter yields a size-independent dimensional constant and thus, allows comparison of organ weights both within and among mammalian species (Stahl, 1965).

Statistical analysis

Means and standard errors were calculated for each group. The means of the measured parameters were subjected to analysis of variance using F ratio and Duncan's New Multiple Range Test (DNMRT) (Fisher and Yates, 1967).

RESULTS

The body weights of control male, castrated male and control female mice were significantly different ($P < 0.01$) when compared using F ratio (Table 1). Following this analysis, further comparison using Duncan's New Multiple Range Test revealed that the body weights of control female mice were significantly smaller than those of control males ($P < 0.01$) and castrated males ($P < 0.01$). There were no significant difference ($P > 0.05$) between the body weights of mice in the control male and castrated male

groups.

The absolute weights of muscles of mice in the three study groups differed significantly ($P < 0.01$) when compared using F ratio (Table 2). Comparison using Duncan's New Multiple Range Test showed that the absolute weights of the muscles of control females were significantly smaller than those of control males ($P < 0.01$) and castrated males ($P < 0.01$). Triceps brachii muscles of castrated male mice weighed less ($P < 0.01$) than those of control male mice. Similarly, biceps brachii, soleus and gastrocnemius muscles of castrated males also weighed less ($P < 0.05$) than those of control male mice.

Analysis of variance using F ratio (Table 3) indicated that there were significant differences ($P < 0.01$) between the muscle mass indices (mg muscle weight/g body weight) of the muscles of mice in the control male, castrated male and control female groups. Application of Duncan's New Multiple Range Test however, revealed that there were no significant differences ($P > 0.05$) between the muscle mass indices of the control females and castrated males. The muscle mass indices of the muscles of control males were greater than those of castrated males ($P < 0.05$) and control females mice ($P < 0.01$).

DISCUSSION

The results of this study demonstrated that the muscles of control male mice were larger than those of control female mice. This observation was evident when the absolute weights of the muscles were

compared, and was confirmed when the muscle mass indices of the muscles were used. This is similar to the reports by previous workers (Goldspink and Rowe, 1968; Row and Goldspink, 1969; Aberle and Doolittle, 1976; Ihemelandu, 1981). The superiority of the muscle mass of males over that of females has been attributed to the stimulatory influence of testosterone on muscle development in the males (Kochakian *et al.*, 1961; Venable, 1966a), as well as to the inhibitory role of oestrogen on muscle development in the females (Ihemelandu, 1980; 1981).

Although the body weights at 12 weeks of age did not differ significantly between control and castrated male mice, castration of weaned male mice in this study played an inhibitory role on the development of muscles. Thus, the muscles of castrated male mice weighed significantly less than those of control males. Furthermore, the muscle mass indices of the muscles of control males were significantly greater than those of castrated male mice. This observation agrees with the report by Ihemelandu and Ibebunjo (1992). Castration has been shown to result in muscular atrophy in cattle (Brannang, 1971; Williamson and Payne, 1975) and red deer (Tan and Fennessey, 1981). The smaller muscle mass of castrated male mice in this study may be due to loss of the stimulatory influence of testosterone on the development of the muscles. Castration will invariably disrupt the hypothalamus – hypophyseal – testis regulatory axis, and so result in elimination of testosterone secretion by testicular interstitial cells of Leydig. Since testosterone has been shown to have a

direct, or at least, an indirect stimulatory influence on muscle development (Venable, 1966a; Goldspink, 1972), elimination of this gonadal steroid through castration will result in loss of any direct effect of the hormone on the muscles, hence, the retardation of muscle growth.

The similarity in the body weights of mice in the control and castrated male groups differs from the observations of Ihemelandu and Ibebunjo (1992). These workers reported that castration of male mice brought about faster growth rate, bigger body size and greater body weight at maturity when compared to intact males. The reason for this disparity is not obvious. However, it may be related to such factors as age at castration and level of nutrition. Indeed, Hale (1973) reported that age at castration may influence growth rate in rats. While Ihemelandu and Ibebunjo (1992) castrated mice at 21 days of age in their study, the mice used in the present study were castrated at 28 days of age.

The absolute weights of muscles of castrated male mice were significantly greater than those of control female mice. It was also evident that the body weights of castrated males were significantly greater than those of control females. However, the lack of any significant difference between the muscle mass indices of the muscles of castrated male and control female mice suggests that the greater muscle weight of castrated males may not be a result of real superiority of their muscle mass. It may rather be due to the greater body weights of the mice in the castrated male group, since the weight of

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any organ is influenced by the weight of the body (Joubert, 1956). The muscle mass index, however, is a size-independent constant that allows comparison of organ weights over a wide range of body sizes (Stahl, 1965), hence, there was no significant difference in the muscle mass indices of the muscles of castrated male and control female mice.

This similarity in the muscle mass of control female and castrated male mice indicates that there was retardation of muscle growth in the castrated male mice. This may have resulted from loss of the stimulatory influence of testosterone on muscle development in the castrated male mice.

In conclusion, this study has demonstrated that castration of male mice gave rise to retardation of muscle growth due to loss of the stimulatory influence of testosterone on the muscles.

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TABLE I: Comparison of body weights (g) at 12 weeks of age using F ratio

Control males	Castrated males	Control females	F value
31.0±0.4	30.4±0.8	23.6±0.4	55.6.*

Values represent Mean ± S.E. for each measurement.

Degrees of freedom = 48. * P < 0.01.

TABLE II: Comparison of absolute weights (mg) of muscles using F ratio

Muscles	Control males	Castrated males	Control females	F value
Biceps brachii	20.8 ± 0.9	18.0 ± 0.7	13.0 ± 0.5	31.2*
Triceps brachii	118.4 ± 2.4	104.6 ± 2.5	78.4 ± 1.7	81.4*
Soleus	7.4 ± 0.2	6.5 ± 0.2	5.1 ± 0.2	27.9*
Gastrocnemius	179.3 ± 3.2	164.5 ± 4.6	126.7 ± 2.6	57.8*

Values represent Mean ± S. E for each measurement.

Degrees of freedom = 48. * P < 0.01.

TABLE III: Comparison of muscle mass indices (mg/g) of muscles using F ratio

Muscles	Control males	Castrated males	Control females	F value
Biceps brachii	0.671 ± 0.025	0.589 ± 0.010	0.549 ± 0.015	14.0*
Triceps brachii	3.830 ± 0.091	3.451 ± 0.077	3.327 ± 0.034	12.4*
Soleus	0.239 ± 0.007	0.215 ± 0.005	0.215 ± 0.037	12.5*
Gastrocnemius	5.791 ± 0.101	5.331 ± 0.090	5.401 ± 0.037	8.8*

Values represent Mean ± S.E for each measurement.

Degrees of freedom = 48. * P < 0.01.